REMARKS

The fee for filing the Request for Continued Examination, the fee for a three-month extension of time and any other fee that may be due in connection with the filing of this paper or with this application should be charged to Deposit Account No. 02-1818. If a Petition for Extension of Time is needed, this paper is to be considered such Petition. A Supplemental Information Disclosure Statement accompanies this response.

Claims 1-11, 13-17, 19-29 and 31-37 are pending. In order to advance prosecution, Claim 1 is amended to replace the recitation "consisting essentially of" with the recitation "consisting of." No new matter is added.

REJECTION OF CLAIMS 1-11 AND 13-17 UNDER 35 U.S.C. § 103(a)

Claims 1-11 and 13-17 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Clark *et al.* (Clin. Microbiol. Rev. 15(3): 329-341 (2002)) in view of Nakamura (Bacteriol Rev 17(3): 189-212 (1953)) and Shimakita *et al.* (US 2003/0003527 Al) and Petri *et al.* (US 5,272,058) because Clark *et al.* teaches a bi-phasic culture medium that allegedly includes every element of the instantly claimed medium except peptone and an antibiotic, and does not teach kits, but Nakamura allegedly teaches media that include peptones and antibiotics, such as penicillin, and Shimakita *et al.* and Petri *et al.* teach kits. The Examiner alleges that it would have been obvious to one of ordinary skill in the art to have modified the media described in Clark *et al.* by adding peptones and antibiotics, based upon the teachings of Nakamura with respect to the art-recognized benefits of adding peptones and antibiotics to culture media, and to include such media in kits as taught by Shimakita *et al.* and Petri *et al.* The Examiner alleges that the result-effective adjustment of particular concentrations of ingredients within the medium, or providing particular ingredients, such as particular antibiotics or peptones, is merely a matter of judicious selection and routine optimization that is within the purview of the skilled artisan.

In maintaining the rejection, the Examiner alleges that

> Clark *et al.* teach the basic composition, an egg slant and a liquid phase comprising salts, while Nakamura teaches peptones, phosphates, and antibiotics, to remove unwanted bacteria such as undesired E. coli. As well, Clark *et al.* teach the creation of axenic medium, "in which the parasite is grown in the absence of any other metabolizing cells" (p. 330).

Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks. The arguments in the previous responses are incorporated herein by reference.

ANALYSIS

It respectfully is submitted that the Examiner has failed to set forth a case of *prima facie* obviousness for the following reasons.

The combination of the teachings of Clark *et al.* and Nakamura and Shimakita *et al.* and Petri *et al.* does not result in the instantly claimed culture medium nor kits

Independent claim 1 and its dependent claims recite a bi-phasic culture medium that comprises a solid phase containing an egg slope or agar slope; and a live Escherichia coli-free liquid phase that consists of: a serum, a peptone, a phosphate buffered saline; and optionally antibiotics. Thus the liquid phase is *E. coli*-free and contains only serum, a peptone, PBS and optionally antibiotics.

Clark *et al.* teaches LE medium or Robinson's medium as the most widely used bi-phasic media for xenic culturing of protozoa. The LE medium described in Clark *et al.* contains 7 recited components in the aqueous liquid phase and the Robinson's medium contains 11 recited components in the aqueous liquid phase. Thus, the cited bi-phasic media described in Clark *et al.* have a liquid phase that contains components that are not recited in the liquid phase of the instant claims. For example, the liquid phase of LE medium contains calcium chloride, magnesium chloride and sodium bicarbonate, and the liquid phase of Robinson's medium includes potassium hydrogen phthalate, citric acid, ammonium sulfate, magnesium sulfate, lactic acid and live standard *E. coli* as ingredients. None of the bi-phasic media of Clark *et al.* has a liquid phase consisting of a serum, a phosphate buffered saline and optionally antibiotics. Clark *et al.* provides no teaching or suggestion to remove the *E. coli* and other additional components.

Nakamura does not cure the deficiencies in the teaching of Clark *et al.* Nakamura teaches the nutrition requirements of the amoeba *Entamoeba histolytica*, including its growth in xenic culture using bi-phasic Locke-egg-serum (LES) medium containing bacteria. Nakamura describes modified bi-phasic media, such as a medium that contains blood agar covered with buffered Ringer-egg white solution. There is no teaching or suggestion in Nakamura to modify the media described in Clark *et al.* by simplifying the liquid phase of the LE medium or Robinson's medium by replacing the liquid phase with a liquid phase consisting of a combination of a serum, a peptone, a phosphate buffered saline and optionally an antibiotic. The only mention of peptone in Nakamura is that peptone has been used for the cultivation of *E. histolytica*, and that peptone may include a growth factor or stimulant for *E. histolytica* (or its bacterial associates). There is no teaching or suggestion in Nakamura that the combination of a serum, a peptone and phosphate buffered saline can replace the

liquid phase of LE medium or Robinson's medium that include live *E. coli*. Further, based on the teachings of Nakamura, one of ordinary skill in the art would not have modified the liquid phase of the prior art media in such a way that the modification would result in the instantly claimed bi-phasic medium. For example, the liquid phase of Robinson's medium and of LE medium contain magnesium ions. The liquid phase of the instant medium does not recite added magnesium ions. Nakamura teaches that modified media were incapable of supporting growth unless magnesium in the form of sulfate, chloride or acetate was added to the medium (page 196). In addition, Nakamura states that "[a]ll the media which were used contained bacteria accompanying the amebas [*sic*]" (page 192). Nakamura also describes attempts in the art to provide media that are bacteria-free, and states that in instances where protozoa were maintained in a "bacteria-free" medium, there was no positive proof that bacteria were completely absent (page 192-193). Thus, none of the teachings of Nakamura would have led one of ordinary skill in the art to have modified the liquid phase of Robinson's medium by removing potassium hydrogen phthalate, citric acid, ammonium sulfate, magnesium sulfate, lactic acid and live standard *E. coli* as ingredients, or to modify the liquid phase of LE medium by removing calcium chloride, magnesium chloride and sodium bicarbonate as ingredients. Thus, the combined teachings of Clark *et al.* and Nakamura do not teach or suggest the instantly claimed medium.

Shimakita *et al.* does not cure these defects. Shimakita *et al.* does not teach or suggest a bi-phasic medium. Shimakita *et al.* describes its culture medium as a solid that contains peptone, sodium chloride, agar and 4',6-diamidino-2-phenylindole dihydrochloride for staining living and dead cells. Shimakita *et al.* does no teach or suggest a liquid medium that consists of a serum, a phosphate buffered saline and optionally an antibiotic. Therefore, Shimakita *et al.* fails to teach or suggest the elements missing from the combined teachings of Clark *et al.* and Nakamura.

Petri *et al.* does not cure the deficiencies in the combined teachings of Clark *et al.*, Nakamura and Shimakita *et al.* Petri *et al.* describes growing a pathogenic strain of *E. histolytica* in monophasic liquid medium TYIS-33 (trypticase yeast extract, iron and serum) with 100 U/ml penicillin and 100 mg/ml streptomycin sulfate. Petri *et al.* also describes growing pathogenic and nonpathogenic strains in monophasic TYSGM-9 medium containing rice starch in the presence of bacterial flora. The only bi-phasic medium mentioned in Petri *et al.* is Robinson's medium. There is no teaching or suggestion in Petri *et al.* to modify Robinson's medium nor to simplify the liquid phase of Robinson's medium nor to eliminate the live *E. coli*

bacteria or any of the ingredients in the liquid phase of Robinson's medium to arrive at the medium as instantly claimed.

There is no teaching or suggestion in the combined teachings of Clark *et al.*, Nakamura, Shimakita *et al.* and Petri *et al.* to simplify a bi-phasic medium by eliminating live *E. coli* bacteria and reducing the number of ingredients in the liquid phase to only serum, a peptone, a phosphate buffered saline and optionally an antibiotic. Therefore, the combination of teachings of Clark *et al.* and Nakamura and Shimakita *et al.* and Petri *et al.* does not result in the instantly claimed medium nor kits. Thus, the Examiner has failed to set forth a *prima facie* case of obviousness of any pending claim.

REBUTTAL TO EXAMINER'S ARGUMENTS

1. **Adding peptones and antibiotics to the liquid phase of the media of Clark *et al.* does not result in the instantly claimed medium.**

Applicant respectfully submits that adding "peptones and antibiotics" to the media of Clark *et al.* "based upon the teachings of Nakamura" does not result in the instantly claimed bi-phasic culture medium. The bi-phasic xenic culture media described in Clark *et al.* are Robinson's medium and LE medium. As discussed above, the liquid phases of these media include ingredients in their liquid phases not recited in the instant medium. For example, the liquid phase of LE medium includes calcium chloride, magnesium chloride and sodium bicarbonate as ingredients and the liquid phase of Robinson's medium includes potassium hydrogen phthalate, bactopeptone, citric acid, ammonium sulfate, magnesium sulfate, lactic acid and live standard *E. coli* as ingredients. Applicant respectfully submits that addition of an antibiotic and/or a peptone to the liquid phase of the bi-phasic media of Clark *et al.* does not result in the liquid phase of the instantly claimed medium because merely adding an antibiotic and/or a peptone does not remove any of the additional ingredients already present in Robinson's medium and LE medium but not recited in the liquid phase of the instantly claimed medium.. Thus, adding peptones and antibiotics to the media of Clark *et al*, "based upon the teachings of Nakamura" does not result in the instantly claimed bi-phasic culture medium.

2. **Nakamura does not teach or suggest "peptones, phosphates, and antibiotics to remove unwanted bacteria such as undesired *E. coli*."**

The Examiner alleges that Nakamura teaches "peptones, phosphates, and antibiotics to remove unwanted bacteria such as unwanted E. coli." Applicant respectfully disagrees. Nakamura teaches the nutrition of *E. histolytica* and describes various attempts to culture this particular species of protozoa. Nakamura identifies the Locke-egg-serum (L.E.S.) medium, a variation of the original Boecke-Drbohlav medium, as being successful for cultivation of the

protozoa. Nakamura recognizes the problem of the available media for cultivating protozoa, stating that "[t]here is a constant search for a simpler medium which requires fewer manipulations and yet maintains the amebas [*sic*] for a long period of time." Nakamura describes various attempts that have been made in the art to provide a simpler medium, most of which resulted in media that were not bi-phasic (page 191-192). Significantly, Nakamura states that "[a]ll the media which were used contained bacteria accompanying the amebas [*sic*]" (page 192). Nakamura also describes attempts in the art to provide media that are bacteria-free, and states that in instances where protozoa were maintained in a "bacteria-free" medium, there was no positive proof that bacteria were completely absent (page 192-193). Nakamura teaches that the "synthetic media" being developed were complex and included live bacteria or live trypanosomes. Nowhere does Nakamura teach or suggest a medium that includes "peptones, phosphates and antibiotics to remove unwanted bacteria such as undesired *E. coli*" as alleged by the Examiner.

Although antibiotics may be used in axenic culture to remove or eliminate bacteria, in xenic culture, antibiotics are not used or are used to reach a balance between the needs of the bacterial flora and those of the protozoa in order for the successful cultivation of the protozoa of interest (see Clark *et al.*, p. 330, col. 2, second paragraph). There is no teaching or suggestion in Nakamura, or any of the cited art, to add an antibiotic to a xenic culture in order to eliminate *E. coli*.

3. Clark *et al.* does not teach or suggest eliminating live *E. coli* from the liquid phase of a xenic culture medium

The Examiner alleges that:

> Clark *et al.* teach the creation of axenic medium, "in which the parasite is grown in the absence of any other metabolizing cells" (p. 330).

While Clark *et al.* teaches that axenic culture media for cultivating some intestinal protozoa in the absence of bacteria exist, Clark *et al.* does not teach or suggest modifying Robinson's medium or any bi-phasic xenic culture medium by eliminating the live *E. coli* from the liquid phase. Instead, Clark *et al.* teaches that axenic culturing requires specially formulated media to replace the nutrients that the protozoa derive from the bacteria in xenic culture. For example, it is taught that axenic culture of *E. histolytica* requires replacement of the bi-phasic LE medium or Robinson's medium (which contains the live bacteria) with any one of monophasic TYI-S-33, YI-S or LYI-S-2 medium, which include no live bacteria but include ingredients not present in Robinson's medium or LE medium, such as yeast extract and vitamins.

The axenic culture media taught in the prior art differs in its components from the instantly claimed medium. Clark *et al.* teaches that the main components of the axenic culture media for protozoa are a source of peptides and amino acids (Trypticase or casein digest peptone), nucleic acids (yeast extract), carbohydrate (glucose), lipids (serum) and vitamins (page 331, paragraph bridging cols. 1 and 2). Clark *et al.* teaches that the components of axenic cultures, such as the yeast extract and vitamins, must provide the nutrients that the protozoa derive from bacteria in xenic culture. The instantly claimed medium does not recite yeast extract or vitamins. Thus, the instantly claimed bi-phasic culture medium is different from all of the axenic culture media described in Clark *et al.*

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In view of the amendment and remarks herein, reconsideration and allowance respectfully are requested.

Respectfully submitted,

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